

The University of Chicago

THE MELANOPHORE HORMONE OF
THE PITUITARY GLAND AND
METABOLIC STIMULATION

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY

1939

By

ROBERT STERLING TEAGUE, Jr.

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THE RELATION OF THE MELANOPHORE HORMONE OF THE PITUITARY GLAND TO OXYGEN CONSUMP- TION OF THE RAT^{1,2,3}

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THE ACTION of the melanophore hormone of the pituitary gland in dispersing melanin granules of melanophore cells in cold-blooded animals is well known and constitutes an important function of the pituitary gland in such animals as the frog (1). However, in higher animals which do not possess chromatophores comparable with those of cold-blooded animals, the function of this hormone remains obscure. Its presence has been demonstrated in the hypophyses of a wide variety of animals, including man (2, 3, 4). Furthermore, its presence in human urine has apparently been demonstrated (5). Thus, one of the important problems connected with the pituitary is the function of the melanophore hormone in warm-blooded animals.

None of the well-known mammalian effects of pituitary extracts has been shown to be due to the melanophore hormone. Zondek and Krohn (6) reported that their purified erythrophore hormone, intermedin (which is biologically similar to the melanophore hormone but apparently different in certain chemical properties), has no action upon growth or upon the gonads. It does not affect blood pressure, or motility of the uterus, bladder, or intestine, and has no antidiuretic effect. Furthermore, it does not influence the blood sugar, nor does it change the glycogen or fat content of the liver. The substance was also reported to have no influence upon basal metabolism. Van Dyke (7) found no change in the fatty acid content of the liver after injection of a beef melanophore hormone extract. The previous reports of antidiuresis in diabetes insipidus with intermedin (8) were shown to be due to contamination with the antidiuretic principle of the neural lobe (9, 10). Schooley, Riddle and Bates (11) stated that in the pigeon intermedin has no power to sustain weight of the crop sac, thyroid, adrenal, testis, pancreas or liver.

The hormone rapidly disappears from the blood stream of animals after intravenous injection and it cannot be found in other tissues (3, 6). Jores (12) in 1933 reported that instillation of melanophore hormone extracts in the human eye facilitated light to dark adaptation of the eye. However, this was refuted by Buschke (13) and has not been confirmed. Jores and Caesar (14) reported that the melanophore hormone dispersed pigment in the retina of the frog's eye and dilated the pupil. On the other hand, Okamoto (15) claimed that hypophysectomy in the toad did not

¹ Aided by a grant from the Otho S. A. Sprague Memorial Institute and Biological Research funds of the University of Chicago.

² Part I of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Chicago.

³ A preliminary report on some of the material given here has been published (TEAGUE, R. S.: *Proc. Soc. Exper. Biol. & Med.* 40: 516. 1939).

affect pigment change in the retina in light and dark adaptation. Jores (16) reported that intermedin and alkaline posterior lobe extracts produced a fall in temperature and a rise in blood sugar in the rabbit. That this hyperglycemic action was actually due to the melanophore hormone is doubtful in view of the negative report of Zondek and Krohn (6). Furthermore, Geiling and Eddy (17) have shown that the hyperglycemia produced by pituitrin fails to appear if the extract is treated with alkali, which destroys the neural lobe principles but potentiates the melanophore hormone. Thus, no unequivocal pharmacodynamic effect of the substance in higher animals has been demonstrated.

However, recently workers in Collip's laboratory have demonstrated that certain pituitary extracts rich in melanophore hormone produced immediate metabolic stimulation in mammals. O'Donovan and Collip (18) showed that a rise in oxygen consumption and carbon dioxide output with a lowering of the R.Q. resulted from injection of their pituitary extracts in the thyroidectomized as well as in normal rabbits. Such effects of various pituitary extracts have occasionally been seen in the past. O'Donovan and Collip, in reviewing the literature suggested that these effects were due to a 'specific metabolic principle' of the pituitary. The observed metabolic changes were attributed to increased fat metabolism and decreased carbohydrate oxidation. The active principle could be distinguished from the thyrotropic, adrenotropic and growth hormones of the anterior lobe, and from the pressor and oxytocic principles of the neural lobe. It was similar to the melanophore hormone in its chemical properties as well as in its anatomical distribution in the hypophysis; hence, its identity with the melanophore hormone was suggested.

Billingsley, O'Donovan and Collip (19) confirmed the metabolic effects of the extracts in rats and guinea pigs. Metabolic stimulation could be obtained in hypophysectomized and adrenalectomized as well as in thyroidectomized animals. Further studies showed that metabolic stimulation of 27% and a lowering of the R.Q. could be obtained in man with the extracts (20).

Preliminary studies (21) indicate that the melanophore hormone accelerates the tyrosine-tyrosinase oxidase system in the production of melanin *in vitro*. Since oxygen was utilized in this reaction, it seemed that Forstvedt's studies might constitute further evidence for the identity of Collip's specific metabolic principle and the melanophore hormone, and at the same time suggest a partial explanation for Collip's findings.

However, there are two reports that prompt further investigation of this subject. Firstly, Zondek and Krohn (6) reported that intermedin did not affect the basal metabolic rate. Secondly, Foster and Smith (22) have shown that the reduction in basal metabolism in the rat after hypophysectomy, was entirely due to removal of the anterior lobe, and not to removal of the pars neuralis and pars intermedia. These workers further found that replacement therapy with posterior lobe extracts (presumably including the pars intermedia) did not raise the basal metabolic rate to normal, as did continued treatment with anterior lobe extracts. Thus it seemed desirable to investigate further the effect of the melanophore hormone on oxygen consumption.

METHODS

In these experiments a study was made of certain pituitary preparations in respect to their melanophore hormone content and to their effect on O_2 consumption in normal, hypophysectomized and thyroidectomized rats. Similar studies were also made of the preparations after destruction or inactivation of the melanophore hormone in various ways. The method for assaying

the melanophore hormone by using the hypophysectomized frog as a test object and alkali-treated U. S. P. XI Posterior Pituitary Powder as the reference standard is described in another communication (23). One unit of the hormone corresponds to the activity of 1 γ of the reference powder.

Preparation of the extracts. Most of the work reported here was done with 3 extracts. One was a purified melanophore hormone extract prepared by Fostvedt (24) from whale anterior lobe⁴ by a modification of Stehle's method (25). Another was a similar extract of beef posterior pituitary⁵ made by the same technic. A partially purified melanophore hormone powder made from beef posterior pituitary, known as 'pitmelanin,'⁶ was also used. These preparations were usually made up in 0.25% acetic acid in a concentration of 10 mg./cc., brought to boil, and filtered. The extracts were sometimes treated with alkali. In this procedure $\frac{1}{2}$ volume of 2N NaOH was added, and the solution was allowed to stand 24 hours at room temperature, and neutralized with 2N HCl with phenol red for the indicator.

Studies were made with some extracts in which the melanophore hormone was inactivated by various means. In all work of this type, control experiments with hormone-inactivated preparations are highly desirable. For destruction of the hormone by acid treatment, an equal volume of concentrated HCl was added to an extract; and it was allowed to stand at room temperature for 24 hours; then it was neutralized with strong NaOH, and adjusted just acid to litmus with 2N HCl. The procedure for tryptic digestion of the melanophore hormone was as follows: to 40 mg. of a pituitary powder there was added 5 cc. of 1% trypsin, 0.5 cc. of 10% Na₂CO₃, and 4.5 cc. of distilled water. The mixture was incubated at 37° C. for 24 hours, 10% acetic acid was added until the solution was just acid to litmus, and the product evaporated over a water bath down to a volume of 4 cc. Ultraviolet light irradiation was used to inactivate the hormone in some experiments. The extracts were placed in a beaker and given 4 to 5 exposures of 60 minutes each to a mercury vapor arc lamp at 30 cm. distance over a period of 2 to 3 days. Inactivation by treating extracts with H₂O₂ was accomplished by simply adding 30% H₂O₂ to the extract and allowing it to stand 24 hours at room temperature. Enough H₂O₂ was added to make a total concentration of 3 to 10%. In every case, the extract was checked for absence of melanophore hormone by injection in a hypophysectomized frog. Extracts of beef liver, muscle and kidney were prepared for control studies by taking up the acetone-dried powder with 0.25% acetic acid at a concentration of 10 mg./cc., boiling 1 minute, and filtering.

Methods of metabolic studies on rats. Both albino and hooded rats were used. The diet consisted of bread, corn meal, powdered milk mixture and raw beef lung, supplemented with lettuce. Hypophysectomy in rats was performed by the parapharyngeal approach, as originally described by Smith (26). Thyroidectomy was carried out with the aid of a dissecting microscope.

⁴ In the whale all the melanophore activity is in the anterior lobe (GEILING, E. M. K.; *Bull. Johns Hopkins Hosp.* 57: 1. 1936).

⁵ Generously supplied by the Wilson Laboratories, Chicago.

⁶ Obtained through courtesy of Parke, Davis & Co., Detroit.

The parathyroids were often removed also, and tetany was prevented by feeding calcium lactate (1%) in drinking water. In both operations, success was judged by a depression of B.M.R. and the operations were checked at necropsy by gross and histologic observations. Autopsies showed that incomplete operation occurred in only one case, that of one of the thyroidectomized rats. At least 3 weeks elapsed before the operated animals were used in the experiments.

Rats both recently fed and fasted 12 to 18 hours were used for O_2 consumption studies. The O_2 consumption was measured in a modified Benedict spirometer with $CaCl_2$ used to absorb the expired water vapor, and soda lime to absorb the carbon dioxide. The container for the rat was an ordinary chemical desiccator which was immersed in a water bath thermostatically maintained at a temperature of $28^\circ C$. Metabolic rates were calculated in terms of liters of O_2 consumed per square meter body surface per 24 hours. The surface area was calculated from the fasted weight using Rubner's formula (27) of $9.1 \times Wt.^{2/3}$.

The usual procedure was to place the rat in the desiccator for 2 to 3 hours for a basal control reading. Thereafter, the rat was removed, injected intraperitoneally, and replaced in the desiccator. A continuous O_2 consumption record was taken for the following 5 to 9 hours. Often the metabolism was again measured the next morning. After a few preliminary trials the rats kept quite still during the greater part of the experiment. In calculating the O_2 consumption, care was taken to utilize only perfectly regular tracings of at least 30 minutes' duration.

RESULTS

The average depression of basal O_2 consumption in the thyroidectomized rats was -23.2% , and in the hypophysectomized rats -34.6% . Comparable results with the extracts were obtained in fed or fasted, normal, hypophysectomized and thyroidectomized rats; that is, an extract possessing metabolic stimulating action would show a response in all types of animals.

In figure 1 are shown the results of control experiments. The highest metabolic rates following injection in each experiment were averaged. The majority of the control results fell within an increase of 10% ; there was, however, considerable variation. In the normal control experiments and in the saline experiments there were 2 cases out of 24 in which the metabolic rise during the day reached $+20\%$. Occasionally with the extracts of liver, muscle, and kidney, an increase up to 30% was seen.

In figure 2(N) is shown a representative graph of the normal metabolic variations during an afternoon, using the figures obtained in the morning as a basis. A typical result with saline injection is essentially similar.

Figure 2 (C) shows the type of response obtained with a large dose (7 to 8 cc./kg.) of Collip's Extract 622⁷ made from beef anterior pituitary and found here to contain 10,000 u/cc. of melanophore hormone. The best results, both as to intensity and regularity of metabolic response, were obtained with

⁷ Obtained through the courtesy of Dr. J. B. Collip.

this extract, which undoubtedly contains a substance that stimulates metabolism. The effect was not obtained with smaller doses as is shown in figure 3 (B and C). These results are similar to those reported by O'Donovan and Collip (18). However, it is significant that a purified melanophore hormone preparation made from whale anterior lobe and assaying 10,000 u/mg., usually failed to produce metabolic stimulation when given in 10 to 20 mg./kg. doses, or 25 times the dose of Collip's Extract 622 (fig. 3, G). In 3 cases there was a rise of over 20%, in 1 case a fall below 30% occurred, while

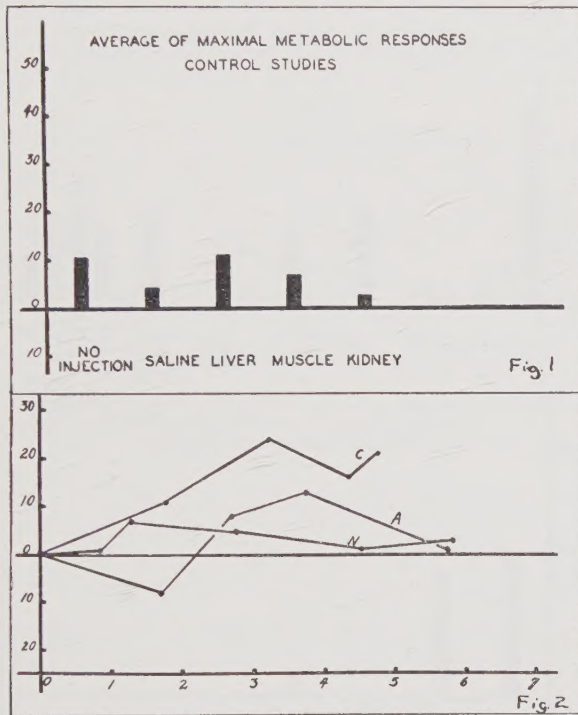


Fig. 1. PERCENTAGE CHANGE IN O₂ CONSUMPTION OVER BASAL VALUE FOLLOWING INJECTION. In experiments with beef liver, muscle and kidney, 10 mg. of each injected. Each average represents at least 7 experiments. Fig. 2. REPRESENTATIVE METABOLIC CHANGES FOLLOWING INJECTION. Abscissa: time in hours following injection at 0. Ordinate: percentage change in O₂ consumption, N, normal control. C, effect of 8 cc./kg. of Collip's Extract 622. A, effect of 8 cc./kg. of Armour & Co. anterior pituitary preparation.

7 other experiments were within a range of normal variation. In these experiments the metabolic response did not parallel the melanophore hormone content of the extracts. This, together with other findings, throws doubt upon the identity of the melanophore hormone and the specific metabolic principle of Collip and coworkers.

An extract⁸ prepared similarly to Collip's Extract 622 containing 2000 u/cc. of melanophore hormone failed to increase the metabolism in doses of 3 to 8 cc./kg., in 5 cases out of 6 (fig. 3, D, E).

⁸ Kindly supplied by Dr. R. L. Kutz.

In another series of experiments, partially purified melanophore hormone made from beef posterior pituitary, assaying 1000 u/mg., was used (fig. 3, F). Doses of 60 to 90 mg./kg. gave a variable response. In 2 cases the metabolic rise was over 20%, but in 3 others there was no response, so that the average rise was less than 20%.

Crude posterior pituitary powder containing 1000 u/mg. of hormone, in a dose of 4 to 8 mg./kg., gave no significant change in metabolism. After alkali treatment in which the posterior lobe principles were destroyed and

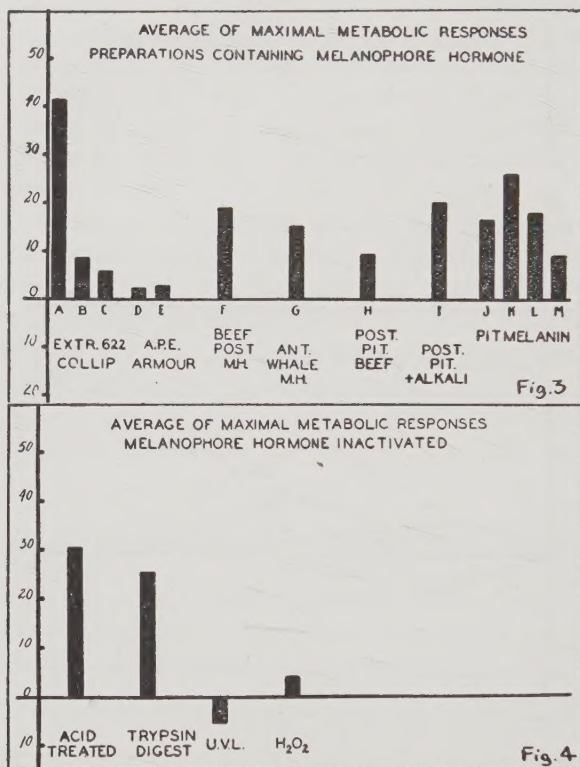


Fig. 3. PERCENTAGE CHANGE IN O₂ CONSUMPTION OVER BASAL VALUE FOLLOWING INJECTION. A, 7 to 8 cc./kg. B, 3 to 4 cc./kg. C, 0.7 to 0.8 cc./kg. D, 7 to 8 cc./kg. E, 3 to 4 cc./kg. F, 60 to 90 mg./kg. G, 10 to 20 mg./kg. H, 4 to 8 mg./kg. I, 4 to 8 mg./kg. J, 200 mg./kg. K, 70 mg./kg. (alkali-treated). L, 70 mg./kg. M, 35 mg./kg. Fig. 4. PERCENTAGE CHANGE IN O₂ CONSUMPTION OVER BASAL VALUE FOLLOWING INJECTION OF INACTIVATED PITMELANIN IN 70 to 150 mg./kg. DOSES.

the melanophore hormone potentiated, injection of the substance in a similar dosage gave one significant change out of 3 experiments (fig. 3, H, I).

Pitmelenin (Parke, Davis & Co.) which is a melanophore hormone powder prepared from beef posterior pituitary, assaying 1000 u/mg., gave insignificant changes in 35 mg./kg. doses (fig. 3, M). With 70 mg./kg. there were 2 increases over 20% and 2 below (fig. 3, L). With the same dosage of alkali-treated material, 3 trials were over 20% and 4 showed no significant change (fig. 3, K). However, on increasing the dose to 200 mg./kg. the response

was lessened (fig. 3, J). The averages likewise show that the only significant response is at 70 mg./kg. level with the alkali-treated material (fig. 3, K). Here, again, the metabolic response failed to follow the melanophore hormone content; hence it cannot be said that the latter is an index to the metabolic stimulating effect of an extract.

The results of injections with inactivated melanophore hormone preparations also did not lend support to the idea that the melanophore hormone and the metabolic stimulating substance are identical.

The averages of these results are shown in figure 4. Destruction of the melanophore hormone was assured in every case by injection into a hypophysectomized frog. Treatment of pitmelanin with concentrated HCl definitely destroyed the melanophore hormone, and yet in 2 experiments an

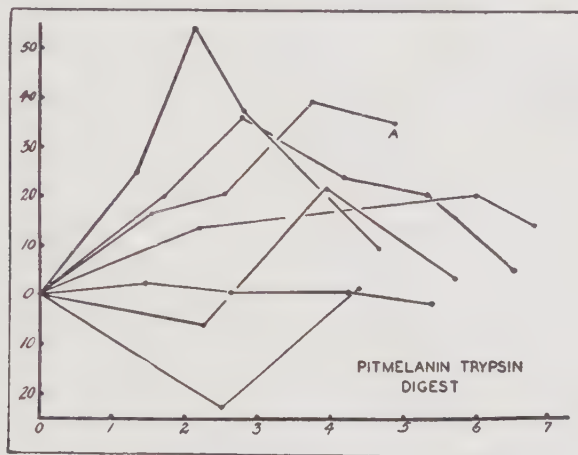


Fig. 5. METABOLIC RESPONSES OBTAINED AFTER TRYPSIN DIGESTION OF PITMELANIN IN 70 TO 150 mg./kg. DOSES. Numerals as in Fig. 2. A, trypsin digest of beef posterior pituitary, 20 mg./kg.

increase in O_2 consumption was still obtained. The resulting product (neutralized) after acid treatment proved toxic to the animals. One of the rats died 1 hour after injection. Trypsin-digested pitmelanin containing no melanophore hormone, produced definite metabolic stimulation in doses of 70 to 150 mg./kg. (fig. 4, 5). It is to be noted that a more consistent metabolic response was obtained with the trypsin-inactivated pitmelanin than with the original extract. In this case, also, metabolic responses were unrelated to the melanophore hormone content. The digested product was not toxic in the dosage used.

After ultraviolet light irradiation of pitmelanin, both the melanophore hormone and the substance responsible for metabolic stimulation are inactivated (fig. 4). The possibility of oxidation by the ultraviolet light treatment was not eliminated, since the extracts were irradiated in open beakers. The resulting product gave an increase of less than 20% on 2 occasions and decreased the metabolism in 8 other trials.

Pitmelanin treated with H_2O_2 likewise lost both melanophore-expanding property and metabolic stimulating effect, as seen from figure 4.

DISCUSSION

In the experiments described above, the only pituitary preparation that consistently stimulated metabolism was Collip's extract. The amount of melanophore hormone contained in the dose of the extract necessary to stimulate metabolism, was entirely out of the (theoretical) physiologic range, 2000 times the amount necessary to darken a frog, when the dose per body weight is compared. Definite indication that the metabolic stimulation produced by Collip's extract is not due to its melanophore hormone content is shown by the absence of a metabolic response with extracts containing equivalent amounts of the hormone, obtained from whale anterior lobe or beef posterior lobe. Furthermore, pitmelanin produced greater metabolic stimulation than did whale anterior lobe preparations, although it contains only 1/10 the melanophore hormone activity of the latter. The fact that the very highly purified whale anterior lobe products, rich in melanophore hormone exhibited the least metabolic effect seems to be good evidence that the metabolic stimulation obtained from pituitary extracts is due to some product other than the melanophore hormone.

Further evidence for this conclusion is found in the experiments with inactivated extracts. More consistent metabolic stimulation was seen with pitmelanin after HCl treatment or tryptic digestion than with the original extract. The toxic action seen in the case of the HCl-treated extract suggests the possibility that the metabolic stimulation produced by certain pituitary extracts might even be non-specific, in that a substance such as a protein breakdown product might be the responsible agent. The finding that ultra-violet light and H_2O_2 treatment destroyed both the melanophore hormone and the substance responsible for metabolic stimulation need not be inconsistent with this suggestion.

During the course of the preparation of this paper there appeared a note by Denstedt and Collip (28), who stated that pituitary fractions had been obtained in which the melanophore hormone and the metabolic principle apparently do not parallel one another. Hence, it can be accepted that the melanophore hormone is not identical with the specific metabolic principle reported by Collip and coworkers. Further search will have to be made to ascertain the rôle of the melanophore hormone in warm-blooded animals. Various efforts are now being made in an attempt to solve this problem.

SUMMARY

Preparations of the pituitary gland rich in melanophore hormone, obtained from various sources and prepared by different methods, vary considerably in their effect on O_2 consumption in rats. Hypophysectomized or thyroidectomized rats are subject to the same variations in response as normal animals.

A pituitary extract of Collip and a melanophore hormone extract pre-

pared from whale anterior lobe, although having a similar melanophore hormone content, showed marked differences in their action on O₂ consumption in rats, in that Collip's extract in large doses was capable of stimulating metabolism while the whale preparation was inactive.

When the melanophore hormone in pitmelanin was destroyed by HCl or by tryptic digestion, the product produced even more consistent metabolic stimulation than the original extract.

The evidence here warrants the conclusion that the melanophore hormone of the pituitary gland is not identical with a substance in pituitary extracts which would increase the B.M.R. This statement finds support in the very recent report of Denstedt and Collip. The data are insufficient to demonstrate the presence of a specific metabolic principle of the hypophysis, since metabolic stimulation was produced with a pituitary extract after treatment with acid and after tryptic digestion, and since metabolic responses were occasionally seen with extracts of muscle, liver and kidney.

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REFERENCES

1. HOGBEN, L. T.: The Pigmentary Effector System, Oliver and Boyd, Edinburgh, 1924.
2. HOGBEN, L. T., AND F. R. WINTON: *Biochem. J.* 16: 619. 1922.
3. HOUSSAY, B. A., AND I. UNGAR: *Compt. rend. Soc. de Biol.* 91: 318. 1924.
4. ZONDEK, B., AND H. KROHN: *Klin. Wchnschr.* 11: 849. 1932.
5. RAZA, S. H., AND W. R. SPURRELL: *J. Physiol.* 93: 429. 1937.
6. ZONDEK, B., AND H. KROHN: *Klin. Wchnschr.* 11: 1293. 1932.
7. VAN DYKE, H. B.: *Arch. f. exper. Path. u. Pharm.* 114: 262. 1926.
8. SULZBERGER, M. B.: *J.A.M.A.* 100: 1928. 1933.
9. SULZBERGER, M. B.: *Klin. Wchnschr.* 15: 489. 1936.
10. FRAZER, A. H.: *J. Pharmacol. & Exper. Therap.* 60: 82. 1937.
11. SCHOOLEY, J. P., O. RIDDLE AND R. W. BATES: *Anat. Rec.* 72: 90. 1938.
12. JORES, A.: *Klin. Wchnschr.* 12: 1599. 1933.
13. BUSCHKE, W.: *Klin. Wchnschr.* 13: 1785. 1934.
14. JORES, A., AND K. G. CAESAR: *Arch. f. d. ges. Physiol.* 235: 724. 1935.
15. OKAMOTO, T.: *Ztschr. f. d. ges. exper. Med.* 101: 155. 1937.
16. JORES, A.: *Ztschr. f. d. ges. exper. Med.* 97: 207. 1935.
17. GEILING, E. M. K., AND C. A. EDDY: *Proc. Soc. Exper. Biol. & Med.* 20: 146. 1928.
18. O'DONOVAN, D. K., AND J. B. COLLIP: *Endocrinology* 23: 718. 1939.
19. BILLINGSLEY, L. W., D. K. O'DONOVAN AND J. B. COLLIP: *Endocrinology* 24: 63. 1939.
20. RABINOWITCH, I. M., M. MOUNTFORD, D. K. O'DONOVAN AND J. B. COLLIP: *Canad. M. A. J.* 40: 105. 1939.
21. FOSTVEDT, G.: *Proc. Soc. Exper. Biol. & Med.* 40: 302. 1939.
22. FOSTER, G. L., AND P. E. SMITH: *J.A.M.A.* 87: 2151. 1926.
23. TEAGUE, R. S.: *Endocrinology*, 25: 962. 1939.
24. FOSTVEDT, G.: Unpublished data.
25. STEHLE, R. L.: *J. Pharmacol. & Exper. Therap.* 57: 1. 1936.
26. SMITH, P. E.: *Am. J. Anat.* 45: 205. 1930.
27. HORST, K., L. B. MENDEL AND F. G. BENEDICT: *J. Nutrition* 3: 177. 1930.
28. DENSTEDT, O. F., AND J. B. COLLIP: *Am. J. Physiol.* 125: 60. 1939.



BIOLOGICAL ASSAY OF THE MELANOPHORE HORMONE
OF THE PITUITARY GLAND

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BIOLOGICAL ASSAY OF THE MELANOPHORE HORMONE OF THE PITUITARY GLAND^{1,2}

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DURING A STUDY of the effect of melanophore hormone preparations upon the oxygen consumption of rats (1), it was found that an accurate assay of the hormone was necessary. Since no established, reliable method for determining its potency has been described, and in view of the recent interest in this hormone, it seemed essential to devise a dependable method and to establish a unit of activity. The hypophysectomized frog was chosen as a test object for the assay, since this preparation has been shown to give a specific response to the melanophore hormone (2). It was found that this subject is somewhat more sensitive than the normal, light-adapted frog, in that skin darkening could be obtained in the hypophysectomized frog with doses that were too small to affect the latter. Furthermore, the response is more reliable in the operated animal, because less control of environmental conditions needs to be exercised. It also seems more physiologic to test the hormone on an intact animal rather than on the isolated frog skin as suggested by Trendelenburg (3). The best results were obtained with frogs (*Rana pipiens*) weighing 30 to 40 gm. Hypophysectomy in the frog is a simple procedure and has been described previously (2, 4).

In all biological assay work it is desirable to compare the activity of the unknown with reference to a standard (5). For this purpose, the United States Pharmacopoeia (U. S. P.) XI Posterior Pituitary Powder was chosen because the method of collecting the glands and preparing the powder makes it probable that the hormone content of this preparation is most constant. The action of the melanophore hormone on frog melanophores is known to be potentiated by at least 10 times when treated with alkali (6). Hence, to avoid confusion, and to detect smaller amounts of the hormone, the reference standard and the unknowns were always treated with alkali.

The method of preparing the standard stock solution is as follows: the posterior pituitary powder is extracted with 0.25% acetic acid and made up to 1 mg./cc. according to the directions in the U. S. P. XI. One-half volume of 2N NaOH is added and the resulting solution allowed to stand at room temperature for 24 hours. Two-normal HCl is added until the solution is just alkaline to phenol red and 0.1N HCl is used to make the solution acid

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² Part II of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Chicago.

to phenol red. Physiologic saline is then added so that the final concentration is $\frac{1}{3}$ mg./cc., and the final pH of the solution is about 6.8. The solution must be kept in the dark at 4° C. With some pituitary preparations a gelatinous precipitate settles out with this treatment. This precipitate is devoid of melanophore activity.

Through extensive trial it was determined that, in general, the injection of 0.001 mg. (1 γ) of the alkali-treated powder produced in about 2 hours in a 30 gm. hypophysectomized frog, a definite submaximal darkening with stellate melanophores, fading in around 6 hours. This amount of the reference standard may conveniently be designated as one unit of melanophore hormone. Some biological variation is encountered, hence at least 6 frogs should be used in an assay. The sensitivity of the method is such that 1/10 of a unit, 0.0001 mg. (0.1 γ) per 30 gm. frog produces no gross color change and rarely any microscopic evidence of melanophore expansion; while 10 U, 0.01 mg. (10 γ) per 30 gm. frog produces a maximal color change, an intense blackening of the skin with completely reticulate melanophores, fading in about 20 hours. Thus for the sake of convenience and in conformity with the rule of Burn (5) that submaximal doses be used in biological assay, a unit of melanophore hormone is defined as the activity of 1 γ (0.001 mg.) of alkali-treated U. S. P. XI Standard Posterior Pituitary Powder, when injected into a hypophysectomized frog in a dosage of 1 γ per 30 gm. frog.

Two important considerations must be observed in the procedure. The frogs tend to become more sensitive to the hormone after 1 to 2 weeks following hypophysectomy. Consequently, better results will be obtained by waiting at least a week after the operation before using the frogs. Furthermore, the frogs must be kept in water at 15° to 20° C. in order to obtain consistent results. At higher temperatures the melanophore reaction is more rapid but the frogs are not so sensitive.

In an assay, the alkali-treated preparations are injected through the floor of the mouth into the ventral lymph sac. In the case of the stock solution, the concentration of $\frac{1}{3}$ mg./cc. makes it convenient to take of a 1:100 dilution, a dose in tenths of a cc., equal to the weight of the frog in gm., in order to give the unit dose of 1 γ per 30 gm. frog. The dilution should be made on the day of the assay. Under the conditions described, the maximal response occurs about 2 hours after injection. Observations of the skin should be made grossly and checked by examination of the web melanophores under a suitable magnification, such as a dissecting microscope. Proper dilutions are made to equate the unknown and the standard at the 1-unit level, and the results are expressed in units, the reference standard containing 1000 U/mg.

The U. S. P. Posterior Pituitary as a dry powder was found to retain its potency over a period of 8 months. A dry purified melanophore hormone preparation containing 10,000 U/mg. was found to deteriorate to 100 U/mg. in the course of 3 months. The standard stock solution in $\frac{1}{3}$ mg./cc. concentration is reliable for only 2 weeks, after which it slowly deteriorates. More dilute solutions are not reliable.

SUMMARY

A method for the biological assay of the melanophore hormone of the pituitary is described, using the hypophysectomized frog as the test object and alkali-treated U. S. P. Posterior Pituitary Powder as the reference standard. A unit of melanophore hormone is defined as the activity of 17 of the alkali-treated standard, when injected into a 30 gm. hypophysectomized frog.

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REFERENCES

1. TEAGUE, R. S.: *Endocrinology*, 25: 953. 1939.
2. TEAGUE, R. S., R. O. NOOJIN AND E. M. K. GEILING: *J. Pharmacol. & Exper. Therap.* 65: 115. 1939.
3. TRENDLENBURG, P.: *Arch. f. exper. Path. u. Pharm.* 114: 225. 1926.
4. HOGBEN, L. T.: *Quart. J. Exper. Physiol.* 13: 178. 1922.
5. BURN, J. H.: *Physiol. Rev.* 10: 146. 1930.
6. STEHLE, R. L.: *Ergebn. der Vitamin-und Hormonforsch.* Band I. Akademische Verlagsgesellschaft M. B. H. Leipzig. 1938.



